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# Transient antiretroviral treatment during acute simian immunodeficiency virus infection facilitates long-term control of the virus

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Experimental evidence and mathematical models indicate that $CD4^+$ T-cell help is required to generate memory cytotoxic T-lymphocyte precursors (CTLp) that are capable of persisting without ongoing antigenic stimulation, and that such responses are necessary to clear an infection or to control it in the long term. Here we analyse mathematical models of simian immunodefiiency virus (SIV) replication in macaques, assuming that SIV impairs specific $CD4^+$ T-cell responses. According to the models, fast viral replication during the initial stages of primary infection can result in failure to generate sufficient long-lived memory CTLp required to control the infection in the long term. Modelling of drug therapy during the acute phase of the infection indicates that transient treatment can minimize the amount of virus-induced immune impairment, allowing a more effective initial immune sensitization. The result is the development of high levels of memory CTLp that are capable of controlling SIV replication in the long term, in the absence of continuous treament. In the model, the success of treatment depends crucially on the timing and duration of antiretroviral therapy. Data on SIV-infected macaques receiving transient drug therapy during acute infection support these theoretical predictions. The data and modelling suggest that among subjects controlling SIV replication most efficiently after treatment, there is a positive correlation between cellular immune responses and virus load in the post-acute stage of infection. Among subjects showing less-efficient virus control, the correlation is negative. We discuss our findings in relation to previously published data on HIV infection.

**Keywords:** simian immunodeficiency virus; treatment; lymphoproliferation; helper cell; memory; immune control

## 1. INTRODUCTION

Experimental simian immunodeficiency virus (SIV) infection in macaques recapitulates many of the features of human immunodeficiency virus (HIV) infection in humans. The ability to control the timing, route and amount of inoculation with characterized virus isolates, and to obtain specimens at defined time points, especially early after inoculation, makes this a uniquely valuable animal model for studies of the dynamic interactions between lentivirus replication and the immune system. As is the case for HIV infection, during the acute phase of experimental SIV infection the virus population typically grows to an initial peak level, followed by a settling of virus load to a somewhat lower post-acute set-point or inflection-point level. This usually marks the beginning of the asymptomatic phase of infection, which eventually culminates in the progressive development of immunodeficiency, characterized by significant depletion of the circulating $CD4^+$ T-cell pool.

Experimental evidence suggests that specific antiviral immune responses, in particular cytotoxic T lymphocyte (CTL), can significantly reduce set-point SIV load (Jin *et al.* 1999; Schmitz *et al.* 1999). The level of set-point virus load has in turn been shown to correlate with the initial growth rate of the virus population (Lifson *et al.* 1997). Hence, the early dynamical interactions between SIV replication and specific immune responses seem to be decisive in determining the level of immunological control achieved in the asymptomatic phase. However, the details of the viral dynamics during acute SIV infection are not fully understood.

The interactions between SIV and the immune system are complex, since $CD4^+$ T cells are not only required to provide help for the specific immune response, but are also a major target cell type susceptible to infection by the virus. Considered mathematically, such interactions are highly nonlinear, resulting in potentially counter-intuitive outcomes. In this context, mathematical models have the potential to take us beyond verbal or graphical reasoning and provide a solid framework on which to generate hypotheses and design experiments. We discuss mathematical models describing the interactions between SIV, $CD4^+$ T cells and the CTL response. We analyse the relationships between the initial rate of virus replication, the degree of immune impairment and the level of

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CTL-mediated virus control. Based on these findings we investigate the effect of drug therapy during the acute phase on the long-term dynamics between the virus and the immune system. Finally we analyse the correlation between set-point virus load and the specific cellular responses. We present experimental data in support of support theoretical predictions.

## 2. VIRUS CONTROL VERSUS IMMUNE IMPAIRMENT

Mathematical models have identified two characteristics of anti-viral CTL responses necessary to clear an infection or to control it in the long term (Wodarz *et al.* 1999*a*,*b*). (i) Reduction of virus load requires a high level of activation and a high proliferation rate of CTL, also termed the 'CTL responsiveness'. This theoretical premise from modelling studies has been supported by genetic data from patients harboring persistent infections such as HTLV-I and HIV-1 (Jeffery *et al.* 1999; Saah *et al.* 1998). (ii) Long-term virus control or clearance requires persistence of memory cytotoxic T-lymphocyte precursors (CTLp) in the face of declining or negligible levels of antigenic stimulation. This ensures that the CTL response maintains pressure on the declining virus population, driving it to extinction. If CTLp do not have the ability to persist without persistent exposure to antigen, the CTL response decays following reduction of virus load. This allows the virus to regain a positive growth rate, eventually equilibrating at a level describing persistent virus replication and chronic productive infection of the host.

In murine virus infections, the development of memory CTLp that are sustained in the absence of antigen has been shown to depend on specific $CD4^+$ T-cell help (Borrow *et al.* 1996, 1998; Thomsen *et al.* 1996, 1998). In the absence of help, inefficient CTL responses develop which lose control of the infection after a given period of time (Planz *et al.* 1997; Thomsen *et al.* 1996, Zajac *et al.* 1998). Both HIV and SIV infection are typically characterized by the absence of significant antiviral $CD4^+$ T-cell responses, especially in the face of the unabated high level viraemia of acute infection (Lifson *et al.* 2000; Rosenberg *et al.* 1999). This absence of help could result in the failure to generate helper-dependent CTL responses that are sustained at low levels of antigen and have the capacity to control viral replication in the long term. This in turn could be one of the factors that contributes to the establishment of persistent SIV or HIV infection and ultimately to the pathogenesis of the immunodeficiency syndrome.

Experimental data on CTL dynamics following the initiation of drug therapy in chronically HIV-infected patients support this hypothesis (Kalams *et al.* 1999). The virus-specific CTL population shows a temporary increase before declining to low levels. The temporary increase in CTL numbers is likely to result from a trade-off between antigenic stimulation and immune impairment. Before virus load has dropped to low levels, absence of viral replication can delay immune impairment, allowing the CTLs to increase. The observation that specific CTLs significantly decline once drug treatment has reduced virus load to low levels indicates that the CTLs seen in SIV or HIV-infected subjects are not long lived at low levels of antigen and hence do not have the capacity to control viral replication in the long term.

## 3. MODELLING IMMUNE IMPAIRMENT IN ACUTE SIV INFECTION

The dynamical interactions between HIV and the immune system can be described by a mathematical model containing four variables: uninfected target cells, infected target cells, CTLp and CTL effectors (CTLe). This model is described in detail in Appendix A. The dynamics between virus and target cells is modelled by the basic virus infection equations (Nowak & Bangham 1996). Here, we specifically assume that the target cells for the virus are immune cells involved in T-cell help, such as CD4 T cells or antigen-presenting cells. We define memory CTLp as $CD8^+$ T cells that have seen antigen, have the capacity to proliferate in response to virus and can persist in the long term. We assume that their expansion depends on the presence of $CD4^+$ T helper cells, and that SIV infection compromises this required helper-cell function. The model does not depend on the specific mechanism(s) underlying SIV-induced helper-cell impairment, except that it is brought about by a fast-replicating virus that reaches a high virus load. CTLe differentiate from CTLp in response to antigen. We assume that CTLe do not have the capacity to proliferate and are relatively short lived. In the model, effector function corresponds to CTL-mediated lysis, although basic conclusions drawn from the model remain similar for models incorporating non-lytic CTL activity (Wodarz & Nowak 1999). Note that we model only memory CTLp responses that depend on $CD4^+$ T-cell help. Less efficient, helper-independent responses are not taken into consideration. Hence, if the model predicts the absence of memory CTLp, this does not necessarily correspond to the complete absence of specific CTL (Wodarz *et al.* 1999*c*).

The model has the interesting property that following viral infection the system may reach one of two equilibria: either (i) a sustained CTL response becomes established and keeps virus load at low levels, or (ii) a sustained CTL response is not established and viral load remains at high levels. The successful development of a CTL memory response depends on host and viral parameters as well as on initial conditions. More specifically, the dynamics between virus and CTL depend on the balance between the rate of viral replication ($\beta$) and the immune responsiveness of the host ($c$ and $b$), factors which influence the virus load attained during primary infection. If the rate of viral replication is below a threshold and is low compared to the immune responsiveness of the host, an efficient, sustained CTL response is always established. On the other hand, if the rate of viral replication is above a threshold and is fast relative to the immune responsiveness of the host, the virus may replicate to high levels and a sustained CTL response fails to become established. For intermediate rates of viral replication, the outcome of the dynamics depends on the initial conditions: a low initial number of CTLp, i.e. the naive state of the host, is likely to result in failure to generate sufficient levels of memory CTLp. This failure is further promoted by a high initial virus load and a low initial $CD4^+$ T-cell count.

Figure 1*a* shows a simulation of primary SIV infection. As simulated in the model, the virus population replicates

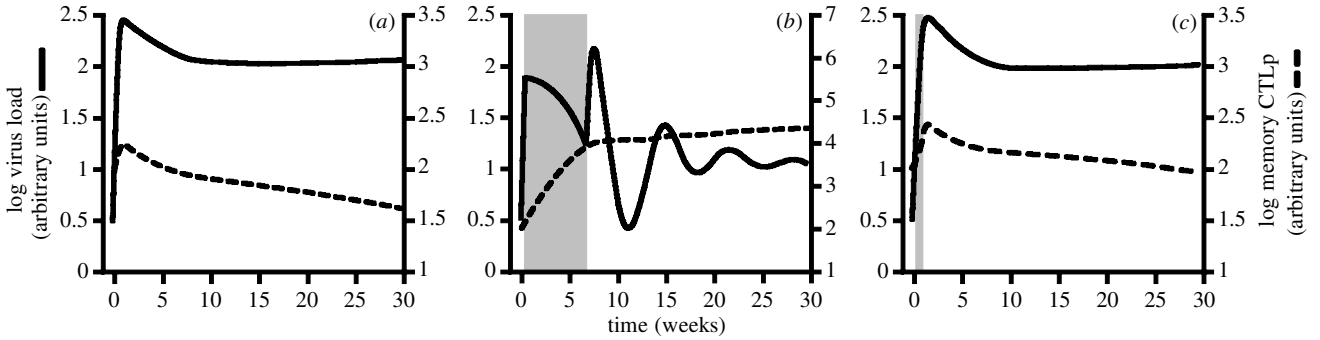


Figure 1. Qualitatively different outcomes predicted by the mathematical model of anti-SIV immune responses (see Appendix A for details). In the model, memory CTLp are defined as CTL precursors that have a relatively long life span even with exposure to low levels of antigen and a high rate of activation. Their generation is dependent on $CD4^+$ T-cell help. The model focuses on the relationship between such memory CTLp and virus control and does not take into account overall CTL dynamics, including inefficient CTLs that do not have the characteristics of memory CTLp and may be maintained by the presence of high levels of persistent antigen during active SIV infection. Shaded areas represent intervals of antiretroviral therapy. (*a*) In the absence of treatment, virus-induced impairment of CD4 cells precludes the development of sustained high levels of memory CTLp, resulting in a lack of efficient virus control. Treatment during the primary phase of infection facilitates establishment of a CTL memory response by inhibiting extensive viral impairment of the helper CD4 responses, while still allowing antigenic sensitization. (*b*) With a sufficiently long treatment period, begun shortly after inoculation, CTL memory is established, i.e. high levels of CTLp persist, facilitating sustained control of viral replication, with damped cycles of viral re-emergence and suppression. (*c*) When brief treatment is begun shortly after inoculation, CTLp develop, but high-level replication of virus emerging after treatment discontinuation results in the absence of CTL memory. Virus load and CTLp were normalized by 2.5 log units and 6 log units, respectively; the time axis was normalized by 3 log units. Simulations shown are based on model parameters as follows: $\lambda = 1$; $\beta = 0.5$; $a = 0.2$; $p = 1$; $c = 0.1$; $b = 0.01$; $q = 0.5$; $h = 0.1$; $s = 0.996$, where $s$ denotes the efficacy of treatment, with $s = 1$ representing 100% efficacy, while $s = 0$% efficacy.

up to a peak and subsequently approaches a stable quasi-equilibrium. In response to antigenic stimulation, the memory CTLp population initially expands, but in the face of virus-induced impairment of T helper-cell function is not maintained at sufficient levels to prevent establishment of persistent infection or to exert substantial control of viral replication in the post-acute phase of infection. As a result, the level of viral replication at quasi-equilibrium is high, and reflective of a progressive infection.

## 4. DRUG THERAPY DURING ACUTE SIV INFECTION

We can introduce drug therapy into the model by assuming that treatment reduces the rate of viral replication (see Appendix A). As shown in figure 1*b*, antiretroviral drug therapy during the primary phase of the infection may result in the establishment of CTL memory and long-term immunological control of the virus following discontinuation of drug treatment. This is because the treatment suppresses viral replication, thereby reducing the amount of T helper-cell impairment during the initial period when the CTL population is expanding in response to antigenic stimulation. By the time that treatment is stopped, the initial conditions have been changed: the virus is now attempting to re-emerge in the face of an established CTL memory response. Because CTLe cells have declined to low levels during treatment, virus load first rises to a peak on discontinuation of therapy. However, CTLp expand and differentiate in response to this antigenic restimulation, enabling elimination of infected cells and reducing viral load. As this cycle is repeated, the system approaches an immune-control equilibrium by damped oscillations. According to the model, treatment during acute infection has fundamentally shifted the balance between virus and host, turning fast progressing dynamics into a state of long-term non-progression.

In the model, the damping time depends on the efficacy of the CTL memory response: prolonged cycling occurs if the CTL memory established is suboptimal. Crucial parameters for successful therapy include the timing of the initiation of treatment, and its duration. If the duration of treatment lies below a critical threshold, virus-induced impairment of $CD4^+$ T-cell function is not sufficiently suppressed and insufficient help is provided for establishment of the CTLp population, resulting in failure to control viral replication in the long term (figure 1*c*).

The duration of treatment required for virus control depends on a number of variables (figure 2). The time when treatment is initiated is an important determinant of success. Starting too early can theoretically result in failure to generate CTL memory because the immune system does not receive sufficient antigenic stimulation. However, this very early treatment might prevent infection in the first place, since replication may be stopped before the virus has disseminated through the body. Starting too late can result in treatment failure because virus replication has already impaired the immune response sufficiently, resulting in failure of CTL memory generation. Other factors that determine the duration of treatment required for success are the replication rate of the virus, the CTL responsiveness, the CD4 T-cell count and the efficacy of the drugs employed for treatment (figure 2).

Representative experimental data from SIV-infected macaques receiving drug therapy during primary infection (Lifson *et al.* 2000) are consistent with our model. As shown in figure 3*a* (Rh 106), in the absence of any

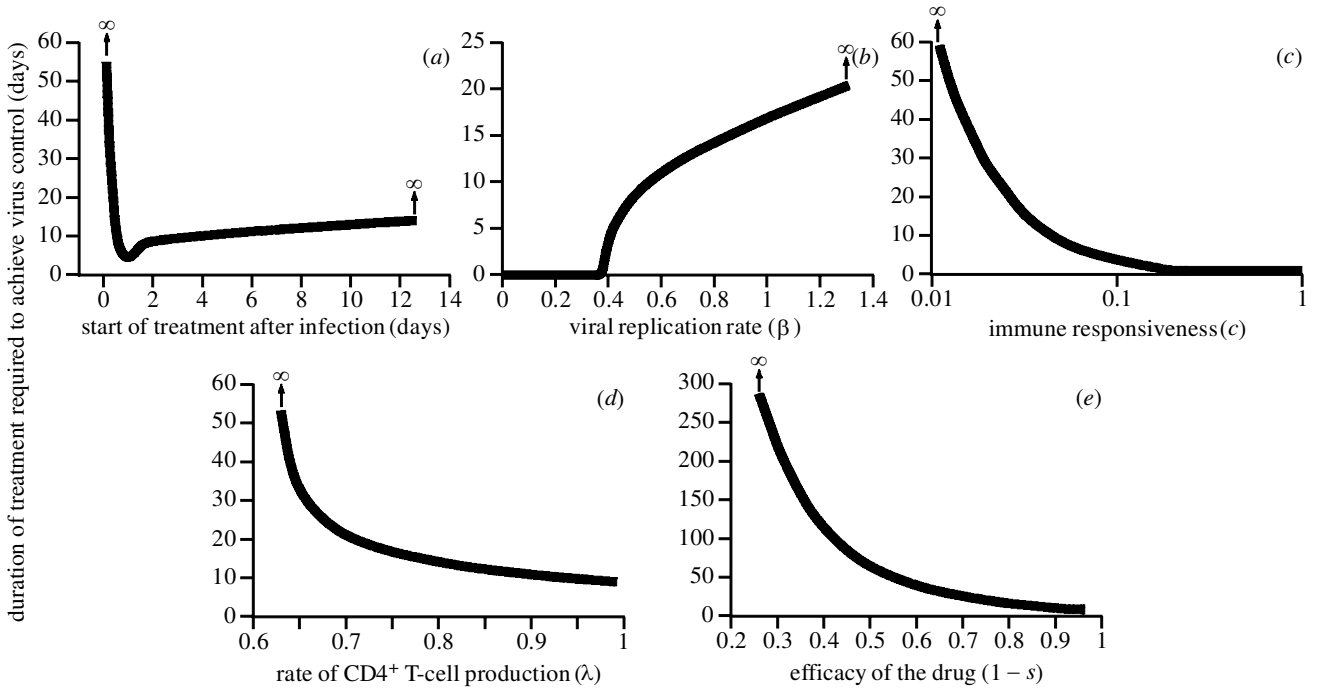


Figure 2. Effects of host and viral parameters on the duration of therapy during primary infection required to establish specific CTL memory. The arrows with an infinity sign denote parameter thresholds beyond which establishment of CTL memory becomes impossible, regardless of the duration of treatment. (*a*) Start of therapy in primary infection or after the drug holiday during the asymptomatic period. Treatment should be started when virus load has replicated to a level sufficient to stimulate specific CTLp. Starting too early may result in treatment failure because the immune system has not been boosted enough. On the other hand, if the virus has replicated to sufficiently high levels, delaying the onset of therapy results in an increased duration of treatment required for the establishment of CTL memory. If treatment is started too late, control of the virus becomes impossible. (*b*) A fast replication rate of the virus, $\beta$, results in a decreased availability of functional T helper cells. Consequently, if $\beta$ lies above a threshold, immune control of the virus is impossible. On the other hand, if the virus replicates relatively slowly and $\beta$ lies below a threshold, virus-induced immune impairment is minimal and the immune system may control the virus without the need for any therapy. For intermediate values of $\beta$, immune impairment interferes with the generation of memory, but therapy may restore it, resulting in long-term immunological control of the virus. In this parameter region, the duration of therapy required to establish CTL memory increases with a faster replication rate of the virus ($\beta$). (*c*) The lower the immune responsiveness of the host ($c$), the longer the duration of treatment required to establish CTL memory. If the immune responsiveness lies below a threshold, treatment cannot result in the establishment of CTL memory. On the other hand, if the immune responsiveness lies above a threshold and is sufficiently high to overcome virus-induced immune impairment, CTL memory is established and the virus is controlled without the need for therapy. (*d*) The rate of $CD4^+$ T-cell production ($\lambda$), and thus the initial $CD4^+$ T-cell count at the start of therapy, is an important parameter for successful treatment. The lower the rate of $CD4^+$ T-cell production, the longer the duration of treatment required to establish CTL memory. If the rate of $CD4^+$ cell production has fallen below a threshold, therapy cannot result in the establishment of CTL memory. (*e*) The efficacy of the drug is denoted by $1-s$, where $s$ ranges from zero to one and represents the degree to which viral replication is reduced. The lower the efficacy of the drug, the longer the duration of therapy required to establish CTL memory. If the efficacy of the drug lies below a threshold, therapy cannot result in the establishment of CTL memory. Baseline parameters were chosen as follows: $\lambda = 1$; $d = 0.1$; $\beta = 0.5$; $a = 0.2$; $p = 1$; $c = 0.1$; $b = 0.01$; $q = 0.5$; $h = 0.1$; $s = 0.0042$.

treatment, viral load increased to a peak value at approximately two weeks post-inoculation, then declined, eventually equilibrating at levels typical of progressive SIV infection ($> 10^6$ SIV RNA copy Eq $ml^1$ of plasma). SIV-specific lymphoproliferative responses, which indicate $CD4^+$ T helper-cell function (see § 5), were notably limited in this animal. In striking contrast, figure 3*b* shows results for an animal in which antiretroviral treatment was initiated 24 h post-inoculation, and continued for 28 days. In this animal, no viral RNA was detected in the plasma during the treatment period, or during a six-week follow-up period following discontinuation of treatment. However, SIV-specific lymphoproliferative responses were demonstrated during the treatment period, indicative of immunological sensitization of $CD4^+$ T cells responding to virus. Continuing cellular immunological sensitization, in the absence of measurable plasma viraemia or seroconversion, probably reflects responses to replicating virus that was present at very low levels, or in an anatomically contained site. When the animal showed no evidence of viral replication during the first six weeks after discontinuation of treatment, it was rechallenged with a second infectious inoculum of the same pathogenic SIV isolate. Strikingly, no plasma virus was detected, although SIV-specific lymphoproliferative responses increased transiently following the rechallenge. These results suggest that the initial treatment regimen had not only prevented the establishment of persistent productive infection, but had also conferred resistance to subsequent direct, intravenous rechallenge with a known infectious

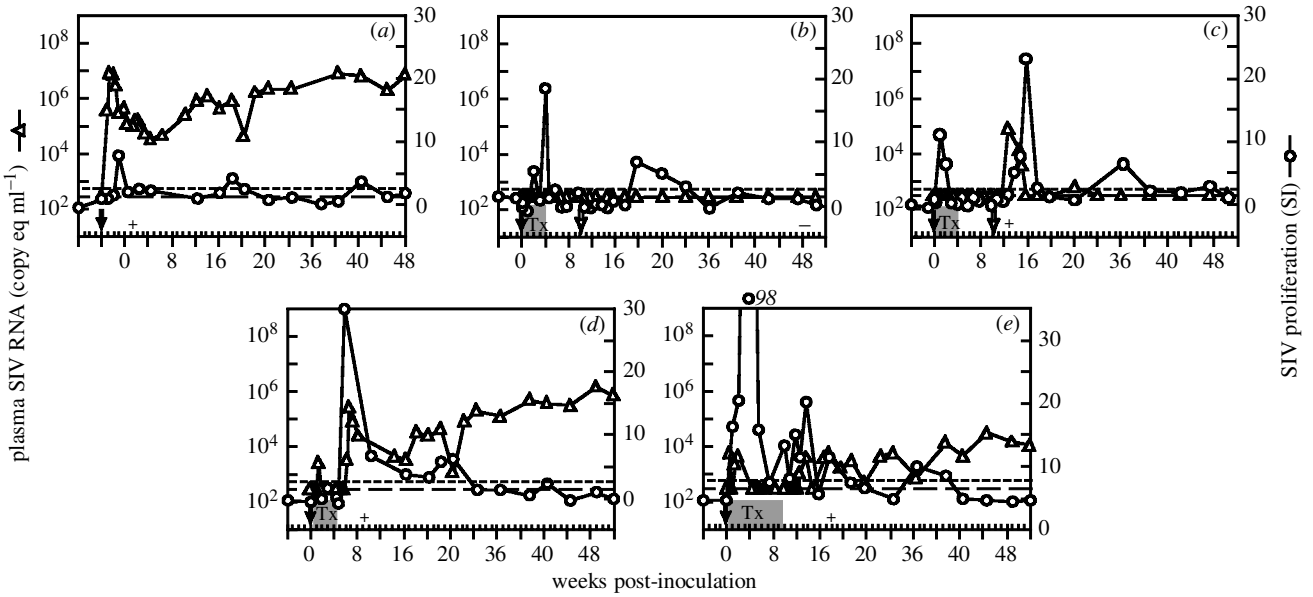


Figure 3. Representative data illustrate different scenarios of viral and host dynamics in SIV infection. Results shown are from the study described in detail in Lifson *et al*. (2000), to which readers are referred for descriptions of experimental procedures. All animal housing and care, and research performed was in conformance with the *Guide for the care and use of laboratory animals* (National Academy Press, Washington, DC 1996). The National Cancer Institute Animal Care and Use Program is fully accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). (*a*) Plasma viraemia (triangles) and anti-SIV proliferative responses (circles, shown as stimulation indices (SI)) for an untreated rhesus macaque (Rh 106) infected intravenously with 20 monkey infectious doses of SIVsm (Lifson & Hirsch 2000). Dashed lines represent the threshold sensitivity of the assay for virion-associated SIV RNA in plasma (300 SIV RNA copy eq ml$^{-1}$; long dashes) and the background of the anti-SIV lymphoproliferation assay (SI < 2.5; short dashes). The solid arrow on the *x*-axis indicated the time of inoculation. The plus sign indicates the time of seroconversion to SIV antigens. Note minimal proliferative responses and poor control of viral replication as reflected in plasma viral RNA levels. (*b*) Results for rhesus macaque (Rh 120) that received 28 days of treatment with tenofovir (9-[2-(R)-(phosphonomethoxy)-propyl]adenine, PMPA, 30 mg kg$^{-1}$, subcutaneously, once daily), beginning 24 h post-inoculation. Graphing conventions are as for (*a*), except that shaded box labelled 'Tx' indicates the interval of tenofovir treatment, the minus symbol on the *x*-axis indicates that the animal did not seroconvert for reactivity to SIV antigens during the study follow-up, and the open arrow on the *x*-axis indicates the time when the animal received a second intravenous challenge with 20 monkey infectious doses of SIVsmE660. Note strong anti-SIV proliferative responses during and after the treatment period, including boosting of proliferative responses following rechallenge, despite the absence of measurable plasma viremia or seroconversion. (*c*) Results for rhesus macaque (Rh 300) that received 28 days of treatment with teonofovir beginning 24 h post-inoculation. Note strong anti-SIV proliferative responses during the treatment period and blunted, self-limited peaks of 'breakthrough' viraemia following rechallenge, with progressive damping of peaks for both plasma viral RNA and anti-SIV proliferative responses. (*d*) Results from a rhesus macaque (Rh 125) that received 28 days of tenofovir treatment, beginning 72 h post-inoculation. Note the transient presence of measurable plasma viraemia during the initial portion of treatment period, and the peak of 'rebound' viraemia following drug discontinuation, associated with strong boosting of anti-SIV proliferative responses. Subsequent loss of control of viral replication (rising plasma RNA levels) was associated with extinction of anti-SIV proliferative response. (*e*) Results for a final animal (Rh 056) that received 63 days tenofovir treatment, beginning 72 h post-inoculation. Note the recurring cycles of blunted peaks of plasma viraemia and proliferative responses, consistent with partial host control of viral replication.

inoculum of a highly pathogenic SIV isolate. Peripheral blood mononuclear cells (PBMCs) from the animal were readily susceptible to SIV infection *in vitro*, demonstrating that absence of productive established infection *in vivo* was not due to any intrinsic resistance to infection at the cellular level.

Figure 3*c* shows a third animal (Rh 300) in which virus was not detected in the plasma following the initial challenge, or in the immediate follow-up period, but was detected following the rechallenge. Strikingly, this post-rechallenge peak was self-limited, declining to below the threshold for measurement, in conjunction with a boosting of anti-SIV proliferative responses. After an additional, even more blunted peak of plasma virus, which declined in conjunction with a boosting of proliferative responses, plasma viraemia declined to below the threshold for measurement and has remained undetectable for months.

Results from two other animals illustrate the potential impact of varying the delay before initiation of treatment, and the duration of treatment. As shown in figure 3*d* (Rh 125), when the same 28-day treatment regimen was not started until 72 h post-inoculation, measurable levels of SIV RNA were detected transiently during the initial portion of the treatment period. While plasma viraemia declined to below the threshold for quantification during the treatment interval, it rapidly re-emerged following drug discontinuation. SIV-specific lymphoproliferative responses were not measured during the treatment period in this animal, but appeared rapidly in conjunction with the peak of re-emergent viraemia following treatment discontinuation. The peak level of viraemia reached after

stopping treatment was lower than that typically seen with untreated primary infection (compare figures 3*a* and 3*d*), and subsequently decreased to below $10^4$ SIV RNA copy eq $ml^{-1}$. As viral load fell, so did SIV-specific proliferative responses; with subsequent increases in viral load, SIV-specific proliferative responses increased. However, with continued follow-up, SIV-specific proliferative responses eventually no longer increased in association with increases in viral load, consistent with cumulative depletion or functional compromise of the responding cell pool. In conjunction with this, viral load rose in a manner that suggested loss of immunological control of viral replication, equilibrating at a level typical of progressive SIV infection (Lifson & Hirsch 2000).

Results from a final animal showed that extending the duration of post-inoculation antiretroviral treatment appeared to compensate, at least in part, for its delayed initiation. As shown in figure 3*e* (Rh 056), delaying the start of treatment until 72 h post-inoculation was again associated with the presence of measurable plasma viraemia during the initial portion of the treatment period, as it was in about half of the total animals evaluated (Lifson *et al.* 2000). With treatment, plasma viraemia declined to below measurable levels, and remained there for the duration of the eight-week treatment period. However, strong SIV-specific proliferative responses were demonstrated during the treatment period. Upon discontinuation of treatment there were several apparent cycles of blunted peaks of re-emergent viraemia that spontaneously declined, often to below measurable levels, without any further experimental intervention. Cyclical peaks and decreases in SIV-specific proliferative responses were also observed, with the system eventually equilibrating at levels of plasma viraemia typically associated with only slowly progressive disease (Lifson & Hirsch 2000), and low to undetectable SIV-specific proliferative responses.

## 5. CELLULAR IMMUNE RESPONSES AND VIRUS CONTROL

In § 4 we established that therapy during acute SIV infection could minimize the amount of immune impairment while allowing more effective antigenic sensitization. This allows for the preservation of an adequate level of $CD4^+$ T-cell help, which is required for the development of a sustained memory CTL response that can control the infection in the long term. Data on SIV-infected macaques confirm this notion, showing that while SIV-specific $CD4^+$ T-cell proliferative responses are generally rare and limited during untreated acute infection, drug treatment increases both the frequency and magnitude of such responses (figure 3; Lifson *et al.* 2000). In this section, we investigate the relationship between the level of CD4 T-cell proliferative responses and virus control once the dynamics converge towards an equilibrium. Previous data on chronically HIV-infected patients showed that loss of virus control was associated with low $CD4^+$ T-cell proliferative responses and low levels of CTL (Ogg *et al.* 1998; Rosenberg *et al.* 1997). Better virus control was associated with higher levels of $CD4^+$ T-cell proliferative responses and CTLs (Ogg *et al.* 1998; Rosenberg *et al.* 1997). Figure 4 shows the correlation between SIV-specific proliferative responses and

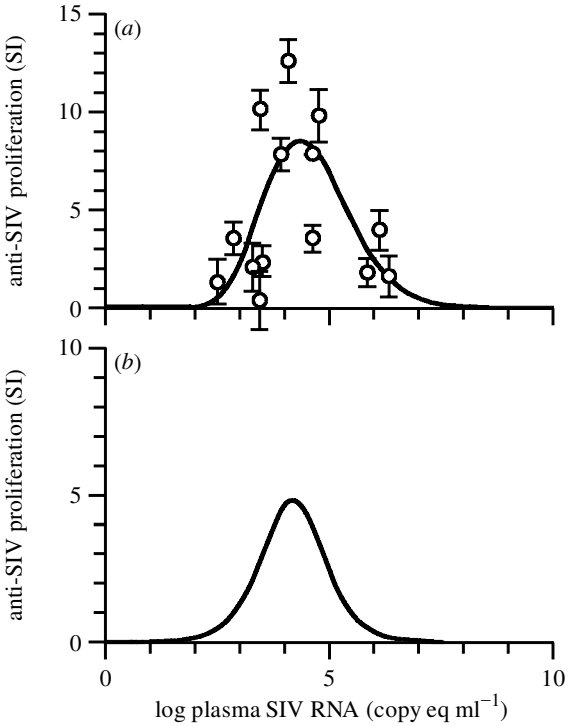


Figure 4. Observed and predicted relationship between viral replication (viral load) and SIV-specific proliferative responses. (*a*) Correlation between experimentally determined values for plasma viral load and SIV-specific proliferative responses. Plotted values are parameter means (mean log for viral load), based on an average of 6.4 data points on viral load and 4.4 data points on the proliferative response for each animal, over an interval beginning 12 weeks following discontinuation of PMPA treatment (or 12 weeks post-inoculation for untreated control animals) and continuing on average for an additional 12 weeks. Means over this interval approximate equilibrium values. Results shown are for all animals except those that were rechallenged (Rh 009, Rh 120, Rh 300; see Lifson *et al.* 2000), and animal Rh 058, which died before this interval. The data suggest a one-humped distribution; this is confirmed statistically with the finding that animals with very low or very high viral loads (averages of $\leqslant 3.75$ or $\geqslant 5.01$ copies SIV RNA $ml^{-1}$ plasma; 33rd and 67th percentiles, respectively; $n = 9$) have significantly weaker proliferative responses than those with intermediate viral loads (averages between 3.75 and 5.01; $n = 5$; $p < 0.0005$). Errors reported are standard deviations of the means. (*b*) Predicted correlation between virus load and $CD4^+$ T-cell responses at equilibrium according to a mathematical model describing *in vivo* replication of HIV in macrophages and T cells. The model is described and analysed in detail in Appendix A.

virus load among the macaques that have been treated during acute infection, with varying degrees of success. We observe a one-humped correlation. Among animals showing the highest levels of virus control and lowest virus load, the correlation is positive. Among animals showing less efficient virus control and higher loads, the correlation turns negative.

To explain these observations, we constructed a mathematical model, taking specific $CD4^+$ T-cell proliferative responses explicitly into account. This modification of the previous model is detailed in Appendix A. We split the $CD4^+$ T-cell population into two subpopulations: resting cells and virus-specific activated $CD4^+$ T cells. Activated

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$CD4^+$ T cells are generated from resting cells and expand in response to antigen. They are responsible for delivering help to the CTL population, and are susceptible to infection by SIV. Based on this model, we can plot the predicted correlation between specific CD4 T-cell proliferative responses and virus load at equilibrium, assuming differences in the degree of immune impairment relative to the rate of antigenic stimulation (figure 4).

The predicted correlation is in agreement with the data from SIV-infected macaques. Loss of virus control is associated with low proliferative responses and high virus load. This is because, under such conditions, the extent of virus-induced immune impairment exceeds the degree of immunity. A decrease in the immune impairment relative to the CTL responsiveness results in lower virus load and higher proliferative responses, as previously observed. However, if the degree of immune impairment falls below a threshold relative to the CTL responsiveness, virus load is reduced even more, and this reduction in virus load is associated with a decrease in CD4 cell proliferative responses. This is because a strong CTL response suppresses virus load to very low levels, removing the amount of antigenic stimulus required to maintain high numbers of specific $CD4^+$ T cells. The positive correlation between proliferative responses and virus load seen in this parameter region may not have been observed in HIV-infected patients because the degree of immunological control in HIV long-term non-progressors may not be as high as that achieved in the SIV-infected macaques treated during primary infection.

To summarize, our data, in conjunction with mathematical modelling, suggest that loss of virus control is associated with high virus load and low proliferative responses, while efficient immunological control of the infection is characterized by low virus load and relatively low, but measurable, proliferative responses. Less efficient immunological control results in relatively high proliferative responses and higher virus load, although the cumulative immune impairment associated with the higher levels of viral replication will eventually diminish the proliferative responses.

Because there is generally an association between the level of $CD4^+$ T-cell responses and the frequencies of CTLs, similar principles should apply to the correlation between CTLs and virus load. Experimental data on HTLV-I infected patients support this notion (Wodarz *et al.* 2000*d*). In agreement with mathematical models, a positive correlation was observed between HTLV-I load and virus load in asymptomatic carriers, who are characterized by efficient CTL-mediated control of HTLV-I load. Mathematical models suggest that the negative correlation observed between virus load and CTLs among HIV-infected individuals (Ogg *et al.* 1998) reflects extensive HIV-induced immune impairment.

## 6. CONCLUSIONS

We have described a theoretical framework which can be used to interpret SIV dynamics during acute infection, and to evaluate treatment regimens aimed at achieving improved immune control of the infection. We conclude that the dynamical processes occurring during acute infection are decisive in determining the quality of the CTL response established. In the natural course of infection, virus-induced helper T-cell impairment can result in the failure to develop and sustain sufficient levels of memory CTLp that are long lived in the absence of ongoing antigenic stimulation. The virus may be temporarily suppressed by less efficient helper-independent CTL responses which eventually lose control of the infection. Treatment during the acute phase minimizes helper T-cell impairment while allowing more effective immunological sensitization. This enables the expansion of memory CTLp to levels that are sufficient for long-term virus control in the absence of continuous therapy. Efficient virus control is characterized by minimal degrees of SIV-induced immune subversion, and this results in a positive correlation between specific cellular immune responses and virus load at the set point. Less efficient virus control results from higher degrees of immune impairment, and this can lead to a negative correlation between cellular responses and virus load.

To test the theoretical framework presented here, it will be necessary to make a detailed analysis of SIV-specific CTL responses. Specifically, the frequency and clonality of specific CTLs should be compared between animals that show different degrees of immune control of SIV replication.

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## APPENDIX A. A BASIC MODEL OF HIV OR SIV INFECTION

To study the relationship between viral replication, viral clearance, and host immune responses, we employed a mathematical model consisting of four variables: uninfected CD4 cells ($x$), SIV-infected CD4 cells ($y$), CTL precursors ($w$) and CTL effectors ($z$). For definitions, see text. The model is given as follows:

$$\left.\begin{aligned} \dot{x} &= \lambda - dx - \beta xy \\ \dot{y} &= \beta xy - ay - pyz \\ \dot{w} &= cxyw - cqyw - bw \\ \dot{z} &= cqyw - hz \end{aligned}\right\}.$$

Uninfected CD4 cells are produced at a rate $\lambda$, die at a rate $dx$ and become infected by free virus at a rate $\beta xy$. Infected cells decay at a rate $ay$ and are killed by CTL effectors at a rate $pyz$. In accordance with experimental findings (Borrow *et al.* 1996, 1998; Thomsen *et al.* 1996, 1998) we assume that establishment of a lasting CTL response depends on $CD4^+$ cell help, and that HIV impairs T helper-cell function. Thus, proliferation of the CTLp population is given by $cxyw$ and is proportional to both virus load ($y$) and the number of uninfected T helper cells ($x$). CTLp die at a rate $bw$ and differentiate into effectors at a rate $cqyw$. CTL effectors die at a rate $hz$. The basic reproductive ratio of the virus is given by $R_0 = \beta\lambda/da$. It denotes the average number of newly

infected cells produced by one infected cell at the beginning of the infection. If $R_0 > 1$, the system may converge to one of two equilibria. The pathogen may replicate in the absence of a CTL response. This is described by equilibrium (E1)

$$x^{(1)} = a/\beta,\ y^{(1)} = \lambda/a - d/\beta,\ w^{(1)} = 0,\ z^{(1)} = 0.$$

On the other hand, a CTL response may be established and this is described by equilibrium (E2)

$$y^{(2)} = \frac{b}{c(x-q)},\ w^{(2)} = \frac{hz^{(2)}}{cqy^{(2)}},\ z^{(2)} = \frac{\beta x^{(2)} - a}{p},$$

where $x^{(2)}$ is given by a solution of a quadratic equation:

$$x^{(2)} = \frac{c(\lambda + dq) - b\beta + \sqrt{[c(\lambda + dq) - b\beta]^2 - 4c^2\lambda qd}}{2cd}.$$

If $cy^{(1)}(x^{(1)} - q) > b$ equilibrium (E1) loses stability and the system converges to equilibrium (E2), i.e. the CTL response invades. If this condition is not fulfilled, equilibrium (E1) is stable. However, equilibrium (E2) may or may not be stable depending on host and viral parameters. If equilibrium (E2) is complex ($[c(\lambda + dq) - b\beta]^2 < 4c^2\lambda qd$) or negative ($x^{(2)} < q$ or $\beta x^{(2)} < a$), the CTL response can never be established. On the other hand, if equilibrium (E2) is positive and real, both equilibria (E1) and (E2) are stable and the outcome depends on the initial conditions. A high initial virus load, a low initial CD4 cell count and a low initial number of CTLp promotes the exhaustion of the CTL response. Overall, a high replication rate of the virus as well as a low immune responsiveness of the host shift the dynamics between SIV and the immune system in the direction of CTL extinction.

### (a) *Including proliferative immune responses*

In order to study the relationship between SIV-specific proliferative responses and virus control at equilibrium, we extend the above model to include both resting and activated CD4 T cells. Activated T cells are generated from the resting cell population specifically in response to SIV infection. Since T cells not specific for SIV can be infected by SIV, and since SIV also infects a variety of alternative cell types, we summarize this target cell pool in a separate variable. The differential equations consist of five variables: resting $CD4^+$ T cells, $s$, uninfected activated SIV-specific $CD4^+$ T cells, $x_1$, alternative uninfected target cells, $x_2$, infected cells, $y$, and CTL, $z$. The extended model is given by the following set of differential equations:

$$\left.\begin{aligned} \dot{s} &= \zeta - fs - rsy \\ \dot{x}_1 &= rsy - d_1x_1 - \beta_1x_1y \\ \dot{x}_2 &= \lambda - d_2x_2 - \beta_2x_2y \\ \dot{y} &= y(\beta_1x_1 + \beta_2x_2) - ay - pyz \\ \dot{z} &= \frac{cx_1yz}{\varepsilon x_1 + 1} - bz \end{aligned}\right\}. \qquad (1)$$

Resting T helper cells are produced at a rate $\zeta$, die at a rate $fs$ and become activated in response to SIV at a rate $rsy$. Activated CD4 T cells die at a rate $d_1x_1$ and become infected at a rate $\beta_1x_1y$. The alternative susceptible target cells are produced at a rate $\lambda$, die at a rate $d_2x_2$ and become infected at a rate $\beta_2x_2y$. Infected cells decay at a rate $ay$ and are killed by CTL at a rate $pyz$. The CTL response grows at a rate $cx_1yz/(\varepsilon x_1+1)$. Since the CTL growth term is proportional to both $x_1$ and $y$, this assumes that CTL expansion not only requires antigen, but also $CD4^+$ T-cell help, and that the virus impairs the immune response. T-cell help is a saturating function of $x_1$, i.e. for very large $x_1$, the rate of CTL expansion approaches $cyz$. Finally, the CTL decay at a rate $bz$.

The basic reproductive ratio of the virus is given by $R_0 = \beta_2\lambda/ad_2$. If $R_0 > 1$, we observe virus replication limited either by target cell availability only, or by a combination of target cell availability and the CTL response. Target cell-limited virus growth is described by equilibrium expressions involving third-degree polynomials and is not written out here. The CTL response becomes established if the immune responsiveness of the host is sufficiently strong relative to the degree of virus-induced immune impairment. More specifically, CTL expansion occurs if $cx_1^*y^*/(\varepsilon x_1^*+1) > b$, where $x_1^*$ and $y^*$ denote the equilibrium values of uninfected activated T helper cells and virus load in the absence of a CTL response. Virus growth controlled by the CTL response is described by the following equilibrium.

Let $A = (d_1c + b\beta_1)(rb + fc)$,
$B = (b[c(\zeta r - f\beta_1 - rd_1) - 2rb\beta_1])$,
$C = rb(b\beta_1 - \zeta c)$,

$$x_1^{(1)} = \frac{B + \sqrt{B^2 - 4AC}}{2A},\ y^{(1)} = \frac{b(x_1^{(1)} + 1)}{cx_1^{(1)}},$$

$$x_2^{(1)} = \frac{\lambda}{d_2 + \beta_2y^{(1)}},\qquad s^{(1)} = \frac{\zeta}{f + ry^{(1)}},$$

$$z^{(1)} = \frac{\beta_1x_1^{(1)} + \beta_2x_2^{(1)} - 1}{p}.$$

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